MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2020

April–June 2020—Volume 135, pp. 245–274

https://doi.org/10.5248/135.245

Pluteus dianae and *P. punctatus* resurrected, with first records from eastern and northern Europe

Hana Ševčíková[1*], Ekaterina F. Malysheva[2], Alfredo Justo[3], Jacob Heilmann-Clausen[4], Michal Tomšovský[5]

[1*] *Department of Botany, Moravian Museum, Zelný trh 6, CZ-659 37 Brno, Czech Republic*

[2] *Komarov Botanical Institute of the Russian Academy of Sciences, RUS-197376, Saint Petersburg, Russia*

[3] *New Brunswick Museum, 277 Douglas Ave, Saint John, E2K 1E5-NB, Canada*

[4] *Centre for Macroecology, Evolution & Climate, Natural History Museum of Denmark, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark*

[5] *Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemědělská 1, CZ-613 00, Brno, Czech Republic*

* Correspondence to: *hsevcikova@mzm.cz*

Abstract—The type specimens of *Pluteus dianae* and *P. punctatus* from the Czech Republic were studied morphologically and molecularly. New collections identified by nrITS sequence analyses extend the distribution of *P. dianae* to Denmark, European Russia, and the Asian part of Turkey and of *P. punctatus* to Sweden. The application of these names is discussed; both belong in the *P. plautus* complex, and data on European and North American taxa in this complex are summarised and compared with *P. dianae* and *P. punctatus*. *Pluteus aestivus* is considered a nomen dubium.

Key words—*Agaricales*, Czechia, *Pluteaceae, Pluteus* sect. *Hispidoderma*, taxonomy

Introduction

Pluteus Fr. (*Pluteaceae*, *Agaricales*) is an agaricoid genus forming basidiomata characterized by free lamellae, a pinkish spore print, smooth globose to ellipsoid (rarely oblong) basidiospores, an inverse hymenophoral trama, and the presence of cheilocystidia and (often) pleurocystidia (Singer 1986, Vellinga 1990). Singer (1986) distinguished three sections in the genus:

Pluteus sect. *Pluteus*, *P.* sect. *Hispidoderma* Fayod, and *P.* sect. *Celluloderma* Fayod (the last comprising *P.* subsect. *Eucellulodermini* Singer and *P.* subsect. *Mixtini* Singer). *Pluteus* sect. *Hispidoderma* is characterized by non-metuloid cystidia and a pileipellis arranged as a trichoderm or hymeniderm (Vellinga & Schreurs 1985, Justo & al. 2011a); *P. dianae* Pilát and *P. punctatus* Wichanský belong in this section. Within the section, according to Pilát (1968), Vellinga (1990), and in concordance with our morphological and phylogenetical studies, both species can be broadly ascribed to the species complex around *P. plautus* (Weinm.) Gillet. Although many species have been described in the *P. plautus* complex, there still exists taxonomic uncertainty regarding the identity of many of its species. Vellinga & Schreurs (1985) placed 13 names in synonymy with *P. plautus*, while other authors who maintained a narrower taxonomic concept (e.g., Orton 1986) accepted several species in this group (e.g., *P. dryophiloides* P.D. Orton, *P. punctipes* P.D. Orton, *P. granulatus* Bres., *P. depauperatus* Romagn.). Recent molecular phylogenies challenge the broad taxonomic concept of Vellinga & Schreurs and emphasize the need for reevaluating names in this complex based on a combination of morphological, ecological, and molecular data (Justo & al. 2011b). In this article we take a first step toward clarifying the taxonomy of the *P. plautus* complex by focusing on type studies of *P. dianae* and *P. punctatus*.

Pluteus dianae was described by Pilát (1968) and *P. punctatus* by Wichanský (1972) based on collections from Czechia (formerly Czechoslovakia). However, no subsequent collections have been reported and no further information on these species has ever been published. Pilát (1968) described *P. dianae* as macroscopically similar to *P. pellitus* (Pers.) P. Kumm., belonging to *P.* subsect. *Depauperati* Lange, a taxon that almost corresponds to *P.* sect. *Hispidoderma* (Lange 1917, Lange 1936, Vellinga & Schreurs 1985). Wichanský (1972) characterized *P. punctatus* by a greyish-brown pileus with dark brown floccose squamules, a whitish stipe densely covered with brown felted squamules, and a pileipellis consisting of very long cylindrical elements.

Materials & methods

Morphology

Macroscopic descriptions of the collected specimens are based on fresh basidiomata. Colour abbreviations follow Kornerup & Wanscher (1983). Morphological terminology follows Vellinga (1988). Microscopic features were studied by H. Ševčíková (holotypes of *P. dianae*, *P. depauperatus*, *P. hiatulus*, *P. inflatus*, *P. puberulus*, *P. punctatus*, and Czech collections of *P. granulatus*, *P. plautus* sensu lato, and *P. semibulbosus* sensu lato) on dried material mounted in Congo red using an Olympus BX-50 light microscope

with magnifications of 400× and 1000×. All Russian collections were studied by E.F. Malysheva using an ammonia Congo red solution under a Zeiss Axio Scope.A1 microscope. Collections studied by A. Justo include material from Denmark, Sweden, and all North American holotypes; microscopic features were studied on dried material mounted in 5% KOH or an ammonia Congo red solution under a Leica DFC2500 microscope. Microscopic descriptions were based on at least 30 measurements each of basidiospores, cheilocystidia, pleurocystidia, and caulocystidia and 25 measurements of basidia. The expression 1/1/30 for basidiospores means 1 collection / 1 basidioma / 30 basidiospores measured in total. Abbreviations: avl = mean of basidiospore length; avw = mean of basidiospore width; avg. = average; Q = quotient of length and width in any one basidiospore; avQ = mean of basidiospore Q-values. Herbarium abbreviations follow Thiers (2018). Authors of fungal names are cited according to the International Plant Names Index website (http://www.ipni.org).

Molecular phylogeny

DNA extraction—The type collections of *P. dianae* and *P. punctatus* were sequenced to establish their position in *P.* sect. *Hispidoderma*. Only dried herbarium specimens were used for DNA extraction. M. Tomšovský extracted the DNA from both holotypes following the protocol of the Qiagen PowerSoil DNA Isolation Kit to step no. 13 (purification of DNA with C2 and C3 buffers) and then continued with MagNA Pure Compact Nucleic Acid Isolation Kit I performed automatically in MagNA Pure Compact Instrument; M. Sochor (Crop Research Institute, Olomouc, Czechia) extracted DNA from the Czech specimens of *P. granulatus* using the CTAB method (Doyle & Doyle 1987); and E.F. Malysheva extracted DNA from the Russian specimens following the manufacturing protocol of the Macherey-Nagel Nucleo-Spin Plant II Kit.

PCR amplification and sequencing—The ITS1–5.8S–ITS2 region from the ribosomal DNA was amplified and sequenced with primer pairs ITS1f–ITS4b or ITS1f–ITS4 (White & al. 1990; Gardes & Bruns 1993) and ITS5–ITS4 (White & al. 1990, Nikolcheva & Bärlocher 2004). PCR products were purified using the Thermo Scientific GeneJET Gel Extraction Kit or by precipitation with polyethylene glycol (10% PEG 6000 and 1.25 M NaCl in the precipitation mixture) and then sequenced with the Sanger method. Raw data were edited and assembled in Sequencher 4.7 and MEGA 6 (Tamura & al. 2013). The sequences generated were deposited in GenBank with corresponding accession numbers (Table 1).

Phylogenetic reconstruction—In addition to 12 ITS sequences newly generated for the study, 77 sequences including the outgroup *Pluteus chrysaegis* (Berk. & Broome) Petch were retrieved for phylogenetic reconstruction from GenBank (http://www.ncbi.nlm.nih.gov/genbank/). The taxonomic identities of these sequences are given as they appear in GenBank (Table 1). The sequences were first aligned with the Muscle method/procedure (Edgar 2004) embedded in MEGA 6 and then corrected manually using the same program. The alignment was deposited in TreeBASE (S23054). Phylogenetic reconstructions for the dataset were performed with maximum likelihood (ML) and Bayesian (BA) analyses. Before the analyses, the best-fit

Table 1. Data for nrITS sequences used in the phylogenetic analyses.
The symbol '!' indicates a type collection.

Taxon	GenBank number (ITS)	Collection (Herbarium)	Geographic origin, ISO code	Source
Pluteus chrysaegis	JN603206	K13587	India	GenBank
P. dianae	**MH656433**	**629413 (PRM!)**	**CZ**	**This study**
	MH656429	**312978 (LE)**	**RU (European part)**	**This study**
	FJ774076	213024 (LE)	RU (European part)	Malysheva & al. 2009
	MH656430	**312950 (LE)**	**RU (European part)**	**This study**
	MH656431	**303485 (LE)**	**RU (European part)**	**This study**
	MH656432	**296318 (LE)**	**RU (European part)**	**This study**
	MH656481	**JHC99-030**	**DK**	**This study**
P. aff. *dianae*	HM562055	AJ209 (CUW)	ES	Justo & al. 2011
P. cf. *fernandezianus*	JQ065028	RSPF 0330	BR	GenBank
P. fibrillosus	KR022018	FK1903 (SP 417462)	BR	Menolli & al. 2015
P. granulatus	**MH656434**	**HS11 (BRNM 807610)**	**CZ**	**This study**
	MH656435	**HS18_2 (BRNM 807611)**	**CZ**	**This study**
	MG544915	OKA-374	TR	GenBank
	MG544916	OKA-349	TR	GenBank
	MG544914	OKA-101	TR	GenBank
	HM562048	AJ203 (LOU)	ES	Justo & al. 2011
	FJ774086	212990 (LE)	RU (European part)	Malysheva & al. 2009
	KR022008	AJ618	US (NY)	Menolli & al. 2015
P. heteromarginatus	HM562058	AJ172 (MICH!)	US (FL)	Justo & al. 2011
P. longistriatus	HM562082	Minnis309203 (SIU)	US (MO)	Justo & al. 2011
	MH211798	61188 (FLAS-F)	US (FL)	GenBank
	MF161220	BHI-F334b (FH)	US (MA)	GenBank
	KM052568	ASIS24529	KR	GenBank
	KX216355	312951 (LE)	RU (Far East)	Malysheva & al. 2016
	MH211936	61504 (FLAS-F)	US (FL)	GenBank
	KY777367	068931 (TENN)	US (TN)	GenBank
	HM562172	394386 (SP)	BR	Justo & al. 2011

Taxon	GenBank number (ITS)	Collection (Herbarium)	Geographic origin, ISO code	Source
	HM562158	393700 (SP)	BR	Justo & al. 2011
	HM562149	394004 (SP)	BR	Justo & al. 2011
	KU953374	-	IT	GenBank
	KT695338	BIOUG24046-H01	CA	GenBank
P. plautus	MG544917	OKA-470	TR	GenBank
	MG544918	OKA-354	TR	GenBank
	KC581304	F23774 (UBC)	CA	GenBank
	KF306033	1859501 (UC)	US (CA)	GenBank
P. aff. *plautus*	KX216322	303680 (LE)	RU (South Siberia)	Malysheva & al. 2016
	KR022015	AJ227	ES	Menolli & al. 2015
	KR022014	A5058325	ES	Menolli & al. 2015
	KR022016	AJ597	US (NY)	Menolli & al. 2015
	KR022026	ACaballero 783	ES	Menolli & al. 2015
	KR022017	163299 (MO)	US (CA)	Menolli & al. 2015
	KR022025	AJ226	ES	Menolli & al. 2015
P. cf. *plautus*	**MH656436**	**HS9 (BRNM 807612)**	**CZ**	**This study**
	MH656437	**HS18_6 (BRNM 807613)**	**CZ**	**This study**
	KJ146724	F23908 (UBC)	CA	GenBank
P. punctatus	**MH656438**	**682743 (PRM!)**	**CZ**	**This study**
	MH656480	**JHC04-298**	**SE**	**This study**
P. romellii	KF306034	1859499 (UC)	US (CA)	GenBank
P. semibulbosus	MG544921	OKA-388	TR	GenBank
	MG544920	OKA-430	TR	GenBank
	MG544919	OKA-362	TR	GenBank
	KR022022	18725 (LOU)	ES	Menolli & al. 2015
	FJ774080	227534 (LE)	RU (European part)	Malysheva & al. 2009
	KX216353	312914 (LE)	RU (European part)	Malysheva & al. 2016
	KR022021	AJ870	ES	Menolli & al. 2015
	KR022023	GM2551	ES	Menolli & al. 2015
	KR022024	FMateo s. n.	ES	Menolli & al. 2015
	MF437007	20150903-15 (SFC)	KR	GenBank

Taxon	GenBank number (ITS)	Collection (Herbarium)	Geographic origin, ISO code	Source
P. semibulbosus (continued)	KF668315	ASIS22533	KR	GenBank
	MG544922	OKA-146	TR	GenBank
P. aff. *semibulbosus*	HM562090	F12393 (TNS)	JP	Justo & al. 2011
P. cf. *semibulbosus*	KR022020	A5058211	ES	Menolli & al. 2015
Pluteus sp.	KC147672	1861124 (UC)	US (CA)	GenBank
	KR022009	93671 (MO)	US (OR)	Menolli & al. 2015
	KR022012	AJ594	US (NY)	Menolli & al. 2015
	KR022013	Miettinen15459	FI	Menolli & al. 2015
	KR022011	AJ606	US (NY)	Menolli & al. 2015
	KR022010	AJ621	US (NY)	Menolli & al. 2015
	KU131672	107524 (PDD)	NZ	GenBank
	KU131673	107527 (PDD)	NZ	GenBank
	KU131674	107530 (PDD)	NZ	GenBank
	KU131675	107529 (PDD)	NZ	GenBank
	KR022019	NMJ193	BR	Menolli & al. 2015
	KC147677	1861231 (UC)	US (CA)	GenBank
	KU131671	107526 (PDD)	NZ	GenBank
	KU131670	107525 (PDD)	NZ	GenBank
	KU131669	107523 (PDD)	NZ	GenBank
	KU131676	107528 (PDD)	NZ	GenBank
	KY352653	055370 (TENN)	AR	GenBank
	KR022028	AJ470	US (VI)	Menolli & al. 2015
	KF306015	1999302 (UC)	US (CA)	GenBank
P. velutinus	KR022027	FK1889	BR	Menolli & al. 2015
	HM562114	F12365 (TNS)	JP	Justo & al. 2011
	HM562127	F12372 (TNS)	JP	Justo & al. 2011
	JN603205	12851 (K)	IN	Pradeep & al. 2012
	KX216340	303693 (LE)	RU (South Siberia)	Malysheva & al. 2016
	MG576118	29375 (MCVE)	IT	GenBank
Uncultured fungus clone	HQ257439	TSBF1_52	BR	GenBank

substitution model for the alignment was estimated based on the Akaike Information Criterion (AIC) using the FindModel web server (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html). Both ML and BA analyses employed the GTR model. ML analysis was run on the PhyML server, v. 3.0 (http://www.atgc-montpellier.fr/phyml/), with one hundred rapid bootstrap replicates. BA was performed using the MrBayes 3.2.5 software (Ronquist & al. 2012) for two independent runs, each with 7 million generations with sampling every 100 generations, under described model and four chains. To quickly diagnose convergence of MCMC analyses and to obtain estimates of the posterior distribution of parameter values, Tracer v1.6 was used (Rambaut & al. 2014). Bootstrap (BS) values ≥70% and posterior probability (PP) values ≥0.95 are considered significant.

Results

Molecular phylogeny

The dataset comprises 88 sequences of *Pluteus* sect. *Hispidoderma* and one outgroup sequence (*P. chrysaegis*) and consists of 693 characters (with gaps). The overall topologies of the ML and BA trees are substantially the same.

The *P. dianae* and *P. punctatus* clades received high statistical support in both analyses. All sequenced collections of *P. dianae* appeared in a single clade in the phylogenetic tree (Fig. 1), including the sequence generated from the holotype collection. Genetic distance between these collections is less than 0.5%. One collection from Spain (AJ209) that appeared extremely close to the *P. dianae* clade but differed in seven nucleotide positions (and with a genetic distance from other collections ca. 1%) is provisionally named *Pluteus* aff. *dianae*.

Collections assigned to the *P. plautus* complex in the sense of Vellinga & Schreurs (1985) did not form a monophyletic clade (Fig. 1). For the time being, we have chosen to assign the published names only to the clades corresponding to *P. granulatus, P. longistriatus, P. semibulbosus,* and *P. velutinus* C.K. Pradeep & al. The other six species sampled here and belonging to the *P. plautus* complex are labelled simply *Pluteus* sp. I, II, III, IV, V, and VI. Work is currently underway to determine which clade should bear the name *P. plautus* and to designate a type that would stabilize the usage of the name.

Taxonomy

Pluteus dianae Pilát, Česká Mykol. 22(3): 171 (1968) Figs 2, 3, 6a,b,d–f

Original description. "Pileus 50–70 mm diam., regulariter orbicularis, adultus plane convexus vel planus, cacumine minime rugoso-reticulatus et ibi tinctu debili luteo-brunneolo, ceterum albus, margine ad dimidium radii rimosus, ceterum laevis glaberque, cutícula marginem in forma membranae tenuissimae superanti, exsiccatus albus tinctu debili sordide isabellino. Lamellae liberae, sat latae et ventricosae, novae

Pluteus sp. KR022012
97/1
Pluteus sp. KR022010
Pluteus plautus KC581304
Pluteus sp. KR022011
Pluteus cf. plautus KJ146724
Pluteus sp. I
89/1
90/0.97
Pluteus sp. KR022013
Pluteus granulatus (HS11)
Pluteus granulatus (HS18_2)
P. granulatus
99/1
Pluteus aff. plautus KX216322
Pluteus aff. dianae HM562055
Pluteus dianae (LE303485)
Pluteus dianae (holotype)
Pluteus dianae (LE312950)
Pluteus dianae (LE296318)
Pluteus dianae (LE312978)
Pluteus dianae (JHC_99_30)
Pluteus dianae (LE213024)
Pluteus plautus MG544918
Pluteus plautus MG544917
Pluteus dianae
92/0.97
100/1
99/1
100/1
Pluteus sp. KC147672
Pluteus sp. KR022009
Pluteus sp. II
99/1
Pluteus velutinus KR022027
Pluteus velutinus JN603205
Pluteus velutinus HM562127
Pluteus velutinus HM562114
Pluteus velutinus MG576118
Pluteus velutinus KX216340
P. velutinus
100/1
Pluteus sp. KY352653
Pluteus sp. KU131669
Pluteus sp. KU131670
Pluteus sp. KU131671
Pluteus sp. III
Pluteus fibrillosus KR022018
Pluteus heteromarginatus HM562058
Pluteus sp. KR022016
Pluteus sp. KR022014
Pluteus plautus KF306033
Pluteus sp. KR022015
Pluteus sp. KC147677
Pluteus romellii KF306034
Pluteus sp. KR022017
Pluteus sp. IV
99/1
Pluteus cf. fernandezianus JQ065028
Uncultured fungus clone HQ257439
Pluteus sp. KR022019
Pluteus semibulbosus MG544922
Pluteus sp. KU131672
Pluteus sp. KU131675
Pluteus sp. KU131674
Pluteus sp. KU131673
Pluteus semibulbosus KF668315
Pluteus semibulbosus MF437007
Pluteus cf. semibulbosus KR022020
Pluteus aff. semibulbosus HM562090
Pluteus semibulbosus MG544919
Pluteus semibulbosus KR022023
Pluteus semibulbosus KR022024
Pluteus semibulbosus KR022021
Pluteus semibulbosus KX216353
Pluteus semibulbosus FJ774080
Pluteus semibulbosus MG544921
Pluteus semibulbosus KR022022
Pluteus semibulbosus MG544920
P. semibulbosus group
74/0.93
73/0.99
83/0.99
78/-
Pluteus longistriatus KU953374
Pluteus longistriatus HM562149
Pluteus longistriatus HM562158
Pluteus longistriatus HM562172
Pluteus longistriatus MH211798
Pluteus longistriatus MF161220
Pluteus longistriatus HM562082
Pluteus longistriatus KT695338
Pluteus longistriatus KY777367
Pluteus longistriatus MH211936
Pluteus longistriatus KM052568
Pluteus longistriatus KX216355
P. longistriatus group
92/0.98
100/1
93/0.98
Pluteus sp. KR022028
Pluteus sp. KU131676
96/1
Pluteus sp. KF306015
Pluteus sp. KR022026
Pluteus sp. KR022025
Pluteus sp. V
99/1
Pluteus granulatus KR022008
Pluteus cf. plautus (HS9)
Pluteus granulatus FJ774086
Pluteus granulatus HM562048
Pluteus cf. plautus (HS18_6)
Pluteus granulatus MG544915
Pluteus granulatus MG544914
Pluteus granulatus MG544916
Pluteus sp. VI
77/1
100/1
Pluteus punctatus (holotype)
Pluteus punctatus (JHC04_298)
Pluteus punctatus
Pluteus chrysaegis JN603206
0.2

albae, adultae albido-salmoneae. Stipes 50–60 × 6–7 mm, albus, minime longitudinaliter rugosus, subcylindraceus, deorsum paulum incrassatus basique truncata ad lignum putridum insidens, parte tertia basali haud raro minime disperse obscurius fibrilloso-subsquamulosus.

"Carposoma, praecipue trama lamellarum et cutis pilei ex hyphis dimiticis, tenuiter tunicatis hyalinis, 4–8 µ crassis et hyphis crassius vel crasse tunicatis, 5–8 µ crassis, conspecte curvatis et haud raro tinctu debili luteolo conspectis, irregulariter et in ángulo recto curvatis et ramosis, parum septatis. Cutis pilei ex hyphis similibus et ex finibus hypharum clavato-saccatis (pileocystidiis) tenuiter tunicatis, hyalinis, decumbentibus, 60–100 × 14–25 µ magnis. Metuloideis crasse tunicatis corniculatisque in hymenio absentibus. Cheilocystidia in acie lamellarum copiosa, ovoidea, saccata vel sublageniformia, tenuiter tunicata, hyalina, 25–60 × 12–25 µ. Sporae globoso-ovoideae, subsalmoneae, laeves, apiculo parvo deflexo praeditae, 7,5–9 × 5–6,5 µ."

Holotypus—Czechoslovakia (currently Czechia), Rozvadov, Diana Nature reserve, in primeval forest, lying rotten trunk of *Fagus sylvatica* L. infected by *Fomes fomentarius* (L.) Fr., 18. VII. 1967 leg. et det. A. Pilát (PRM 629413).

Type revision. Basidiospores [1/1/30] (6.0–)6.5–8.5(–9.5) × 5.0–6.5(–7.0) µm, avl × avw = 7.2 × 6.3 µm, Q = 1.00–1.40(–1.60), avQ = 1.16, globose, subglobose or broadly ellipsoid, rarely ellipsoid. Basidia (18–)21–29(–32) × 7–9(–11) µm, tetrasterigmate, narrowly clavate to subcylindrical or subutriform, colourless. Pleurocystidia moderately abundant, (32–)35–82(–90) × (13–)15–26(–32) µm, fusiform, lageniform, less frequently utriform, usually with a ≤15 µm (very rarely ≤35 µm) long narrowed apex and 3.0–15.0 µm long pedicel, thin-walled, colourless. Lamellar edges sterile. Cheilocystidia (25–)30–48(–62) × (12–)14.5–26 µm, narrowly to broadly clavate, narrowly ventricose, scarcely sublageniform or cylindrical, thin-walled, colourless. Pileipellis a trichoderm, terminal elements 55–180(–280) × 14–24 µm, very variable in size, sometimes in similarly sized tufts (more often smaller than larger-sized elements), narrowly clavate to clavate, sometimes with a narrowed or strangulated apex, sometimes with pale ochre intracellular pigment. Branched hyphae present in the subpileipellis. Stipitipellis a cutis of 4.0–12.0(–18.0) µm diam. cylindrical colourless hyphae. Caulocystidia in tufts, (30–)45–75(–82) × (9–)12–17.5(–20) µm, cylindrical or narrowly clavate, rarely with short obtuse rostrum, thin-walled, colourless or with very pale intracellular pigment. Clamp connections absent in all studied tissues.

Fig. 1. Best tree from Maximum likelihood analysis for the nrITS dataset showing some lineages within *Pluteus* sect. *Hispidoderma*. For sequences retrieved from GenBank the corresponding accession numbers are given. The newly generated sequences are indicated with the collection numbers (in parentheses). Support values given above the branches are BS ≥70% /PP≥0.95. Scale bar shows expected changes per site.

Characteristics of the type locality and type specimen

Diana Nature Reserve is a protected area in western Czechia close to the German border and situated in the Český les mountains near the village of Rozvadov at 500–532 m a.s.l. (central point: 49°37′57″N 12°34′43″E). The Český les mountain range belongs to the Bohemian massif Mesophyticum (Skalický 1988) with a ca. 7–8 °C mean annual temperature and 700–800 mm mean annual precipitation. Climatically, this region is classified as mildly warm zone (MT3 on the Quitt scale, Vrška & al. 2012). The reserve comprises predominantly near-natural herb-rich beech forests, in particular the *Dentario enneaphylli-Fagetum* association in an advanced stage of degradation (Zahradnický & Mackovčin 2004). Based on their mensurational surveys, Vrška & al. (2012) detected an extensive disturbance in a part of the reserve, thus establishing that Diana is not now a natural forest. The protected area comprises a 300-year old forest of *Tilia cordata*, *Quercus robur*, and *Picea abies*. Pilát (1968), who collected the holotype collection from a rotting trunk of *Fagus sylvatica* lying on the ground, stated that he collected and photographed two basidiomata. However, only one basidiocarp is shown in the photograph (Pilát 1968: 172). The original material is preserved in dried form and in good condition in the herbarium of the Mycological Department of the National Museum in Prague (PRM 629413!).

Additional collections studied

Pileus 20–120 mm in diam., broadly conical, convex to applanate, sometimes with broad low umbo; creamy-grey or cinnamon buff, greyish-brown or brown; drying ± whitish-grey, surface felty or covered with white appressed hairs; in some specimens irregularly wrinkled in centre, in some specimens striate at margin. Lamellae pink with concolorous edges. Stipe 30–70(-100) × 2.5–10 mm, broadened towards base or bulbous; pure white, whitish, or yellowish; longitudinally striate, whitish floccose to granulose-pruinose. Smell indistinct or pleasant, sweetish-spicy.

Basidiospores [5/5/150] 5.5–8.0(-9.0) × 4.5–6.5(-6.8) µm, avl × avw = 7.2 × 5.5 µm, Q = (1.10-)1.17–1.46, avQ = 1.30, broadly ellipsoid to ellipsoid or ovoid, rarely subglobose. Basidia 25–37 × 8–12 µm, tetrasterigmate. Pleurocystidia abundant, 54–86(-103) × (14-)18–27(-34) µm, mostly (broadly) lageniform, (narrowly) utriform, or fusiform, commonly with a small apical excrescence, more rarely with more than one on the same cystidium, colourless. Cheilocystidia abundant, forming a sterile layer, very variable in size, (41-)52–95(-115) × (14-)17–27(-32) µm, narrowly

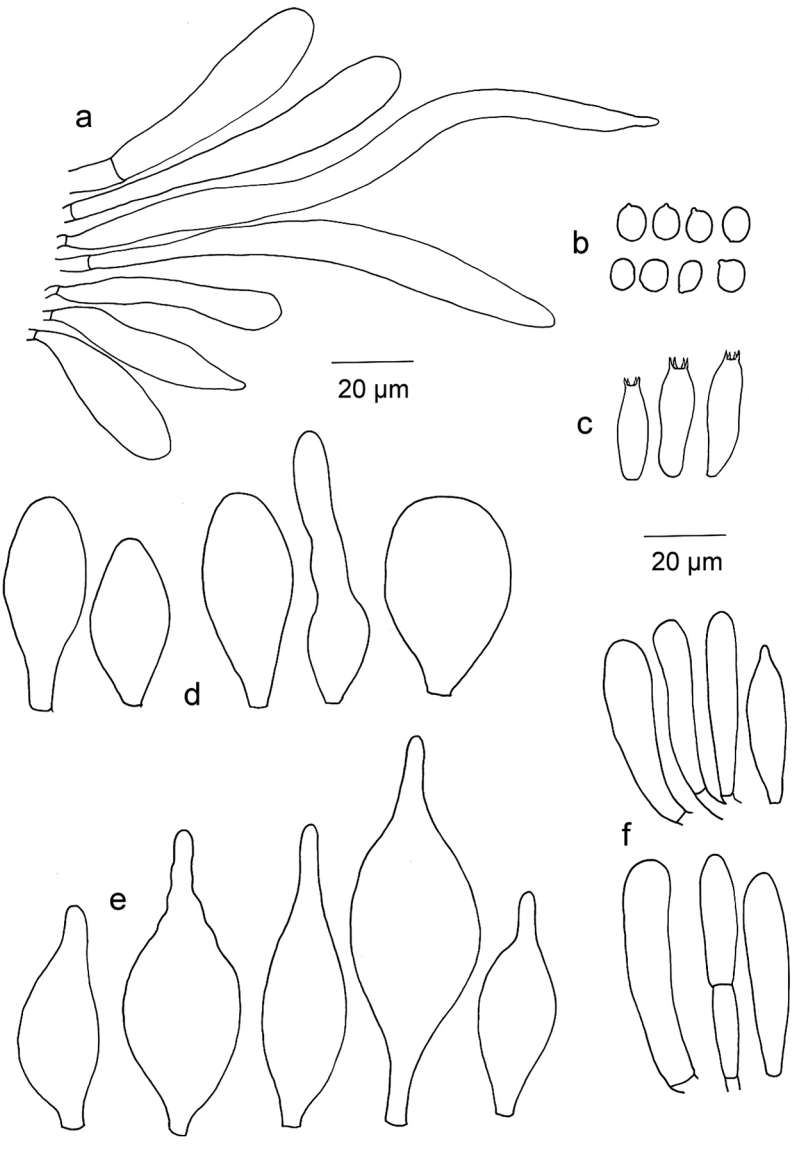


Fig. 2. *Pluteus dianae* (holotype, PRM 629413): a. pileipellis elements; b. basidiospores; c. basidia; d. cheilocystidia; e. pleurocystidia; f. caulocystidia. (del. H. Ševčíková).

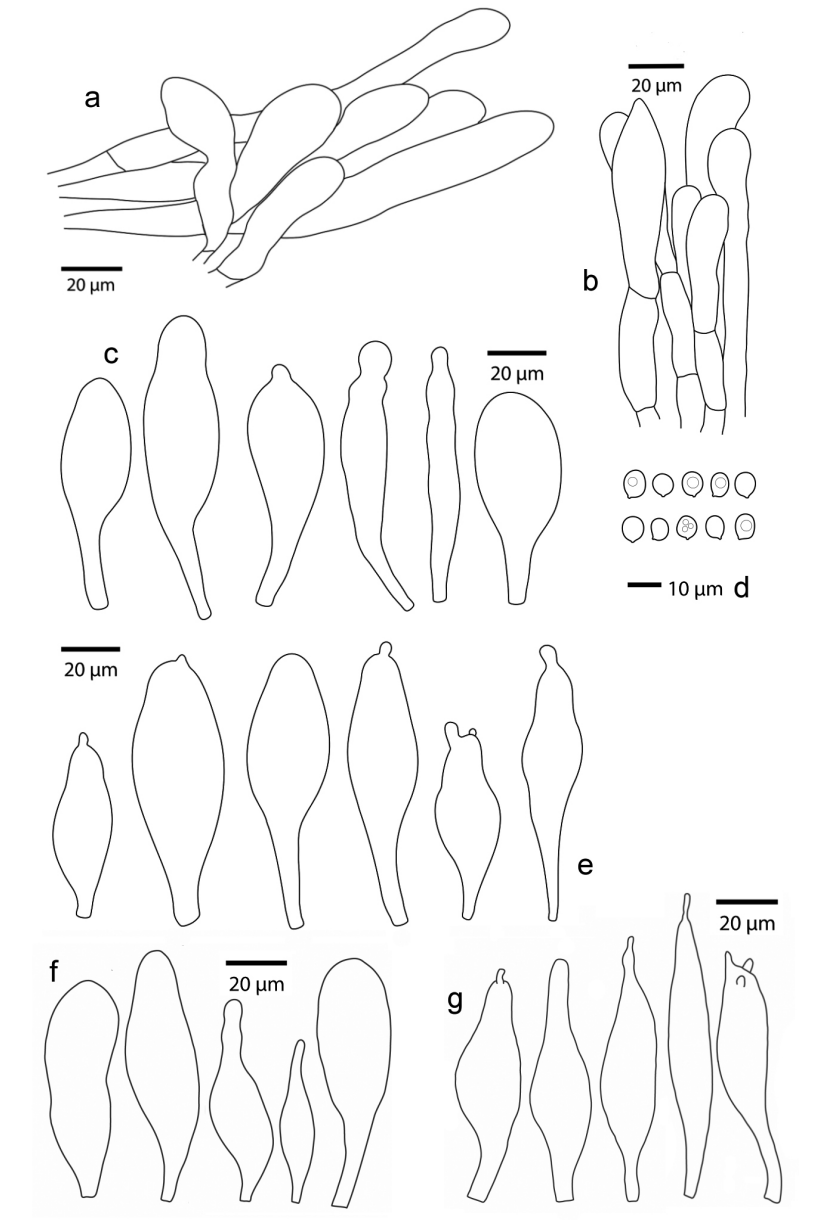


Fig. 3. *Pluteus dianae* (LE312950): a. pileipellis elements; b. caulocystidia; c. cheilocystidia; d. basidiospores; e. pleurocystidia. (del. E.F. Malysheva); (JHC 99_30): f. cheilocystidia; g. pleurocystidia. (del. A Justo).

to broadly clavate, narrowly utriform to utriform, ovoid or cylindrical, lageniform, pedicellate, some with single apical papilla, thin- or slightly thick-walled. Pileipellis a trichoderm, terminal elements (50–)79–125(–220) × 14–36 µm, clavate, utriform or cylindrical, some with tapering apex. Caulocystidia in clusters, 43–140 × 14–27 µm; narrowly clavate, narrowly utriform, narrowly fusiform, or narrowly lageniform; thin-walled, colourless. Clamp connections absent.

Collections studied—**DENMARK**, **Sjælland**, Næsbyholm Storskov [SW of Ringsted], 55°22'26"N, 11°35'50"E, on fallen log of *Fagus sylvatica*, 24 VIII 1999, leg. J. Heilmann-Clausen (JHC99-030, as *P.* cf. *depauperatus*). **RUSSIA**: **Samara Region**, Zhiguli State Nature Reserve, broadleaved forest (*Tilia cordata*, *Acer platanoides*), on fallen trunk of deciduous tree, 9 VII 2003, leg. E. Malysheva (LE 312950, as *Pluteus plautus*); broadleaved forest, on fallen trunk of deciduous tree, 27 VIII 2001, leg. E. Malysheva (LE 312978, as *Pluteus plautus*); **Moscow Region**, Zvenigorod Biological Station of Moscow State University, 55°41′00″N 36°43′00″E, mixed forest, on fallen trunk of *Populus tremula*, 19 VI 2013, leg. E. Voronina (LE 296318, as *Pluteus plautus*); *Picea* forest (with *Pinus* and *Betula*), on decayed deciduous tree, 10 VI 2014, leg. E. Voronina (LE 303485, as *Pluteus plautus*).

Comments—The Danish collection was from a somewhat open, seminatural, deciduous woodland dominated by *Fagus sylvatica* and on a north-facing slope facing the river Susåen. The annual temperature of the site averages 8.6–8.8 °C, and the annual precipitation averages 600–700 mm (Wang 2013). The Russian collection sites lie in the submontane belt and mountain territory (Zhiguli State Nature Reserve) and plains (Moscow State University Zvenigorod Biological Station) with the annual temperature averaging 10.3 °C and the total annual precipitation averaging 904.4 mm (http://www.earthonlinemedia.com/ipg/outlines/climates_humid_continental.html).

Pluteus punctatus Wichanský, Mykol. Sborn. 49(1–2): 1 (1972) Figs 4, 5, 6c

Original description. "Pileus tenuiter carnosus, 5.5 cm diam., pallide griseo-brunneolus, late convexus, dimidio squamulis brunneis minutis flocculosis punctiformibus ad marginem absentibus obtextus. Lamellae confertae, ventricosae usque 10 mm latae, remotae, albidae, dein incarnatae. Stipes 4 cm longus atque 8 mm crassus, solidus, cylindraceus, ad basim paulum incrassatus, albus, nudo apice excepto aquamulis brunneis, minutis, flocculosis dense punctatus. Cystidia varie formata, ellipsoideo- vel ovato-clavata, nonnullae ventricoso fusiformia. Sporae late ellipsoideae, 7–8.5 × 5.5–6.5 µm. Cellulae cuticulae pilei cylindricae, longissimae, 13–26 µm crassae. Caro pilei et stipitis alba. Odor et sapor nullae."

Holotypus—Carposoma solitarium do. Frant. Fuchs 4. X. 1969 ad codicem *Populi albae* in Řevnice (Czechoslovakia [currently Czechia], Bohemia centralis) legit et mihi donavit. Typus in herbario Musei nationalis Pragae asservatur (PRM 682743).

Type revision. Basidiospores [1/1/30] (5.0–)7.0–8.5(–9.5) × 5.0–7.0(–7.5) µm, avl × avw = 7.6 × 6.5 µm, Q = (1.00–)1.05–1.33(–1.40), avQ = 1.2, subglobose or broadly ellipsoid, rarely globose or ellipsoid. Basidia (18–)22–28 (–30) × 8–10(–12) µm, tetrasterigmate, rarely bisterigmate, narrowly clavate to subcylindrical or subutriform, colourless. Pleurocystidia scattered, 28–65 × (13–)15–26(–32) µm, fusiform, lageniform, less frequently utriform, often with ≤9 µm long obtuse apex, sometimes with ≤13 µm long pedicel, thin-walled, colourless. Lamellar edges sterile. Cheilocystidia abundant (28–)44–65 (–86) × (14–)16–33 µm, broadly clavate, narrowly ventricose, fusiform to broadly fusiform, obovoid, scarcely sublageniform, sometimes with ≤12 µm long pedicel, rarely with a short rounded apex, thin-walled, hyaline. Pileipellis a trichoderm, terminal elements 55–280(–380) × 13–26(–38) µm, variable in size, narrowly clavate to clavate, sometimes with a narrowed or slightly strangulated apex, with or without colourless or with pale brown intracellular pigment. Stipitipellis a cutis of 5.0–11.0(–16.0) µm diam. cylindrical hyaline hyphae. Caulocystidia solitary or in small tufts, scattered, (24–)28–60 × 10–25 µm, cylindrical or narrowly clavate, obovoid with short pedicel or obfusiform, thin-walled, with or without very pale brown intracellular pigment. Clamp connections absent in all studied tissues.

Characteristics of the type locality and type specimen

Řevnice is a town situated about 10 km southwest of the Prague city limits on the Berounka river, lying at 200 m a.s.l. (surrounded by ca. 500 m hills (central point: 49°54′50″N 14°14′09″E). The area belongs to the Bohemian Massif, Thermophyticum (Skalický 1988) with annual averages of 8.5–9 °C and 504–527 mm precipitation (Merkel 2018, Tolasz 2007). Wichanský (1972) did not describe the ecology in detail, citing only the growth on a *Populus* sp. stump. Therefore it is unclear whether the type specimen was found in a semi-natural alluvial woodland, a disturbed stand, or an ornamental tree in the town.

Wichanský (1972) mentions only one basidiocarp, and no other original material exists. This basidiocarp is preserved as a holotype in the herbarium of the Mycological Department of the National Museum in Prague (PRM 682743 contains one dried basidiocarp in good condition).

Additional collection studied

Pileus scales greyish-brown in centre, outwards cream to ochraceous, ≤ 25 mm diam. (dried specimen). Lamellae free, 1–2 tiers of lamellulae, crowded, lamellar edge concolorous. Stipe 32 × 2 mm (dried specimen), cylindrical, broadened towards base, finely pruinose. Smell and taste not recorded.

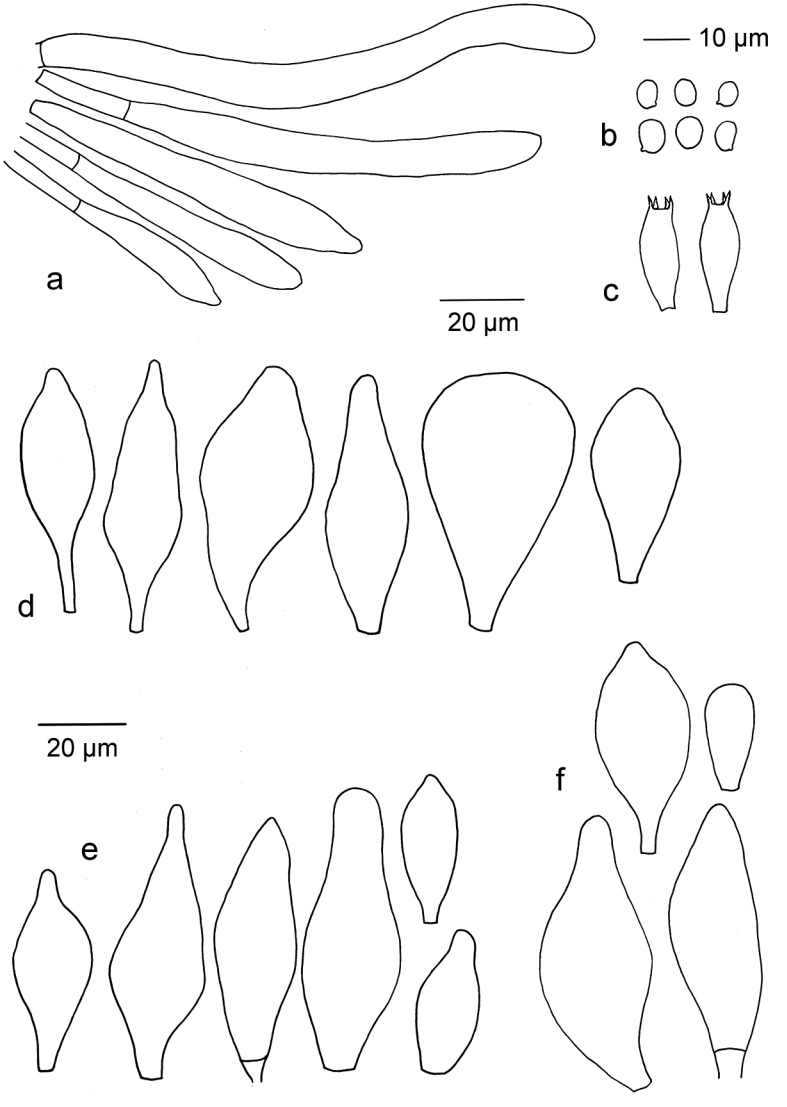


Fig. 4. *Pluteus punctatus* (holotype, PRM 682743): a. pileipellis elements; b. basidiospores; c. basidia; d. cheilocystidia; e. pleurocystidia; f. caulocystidia.

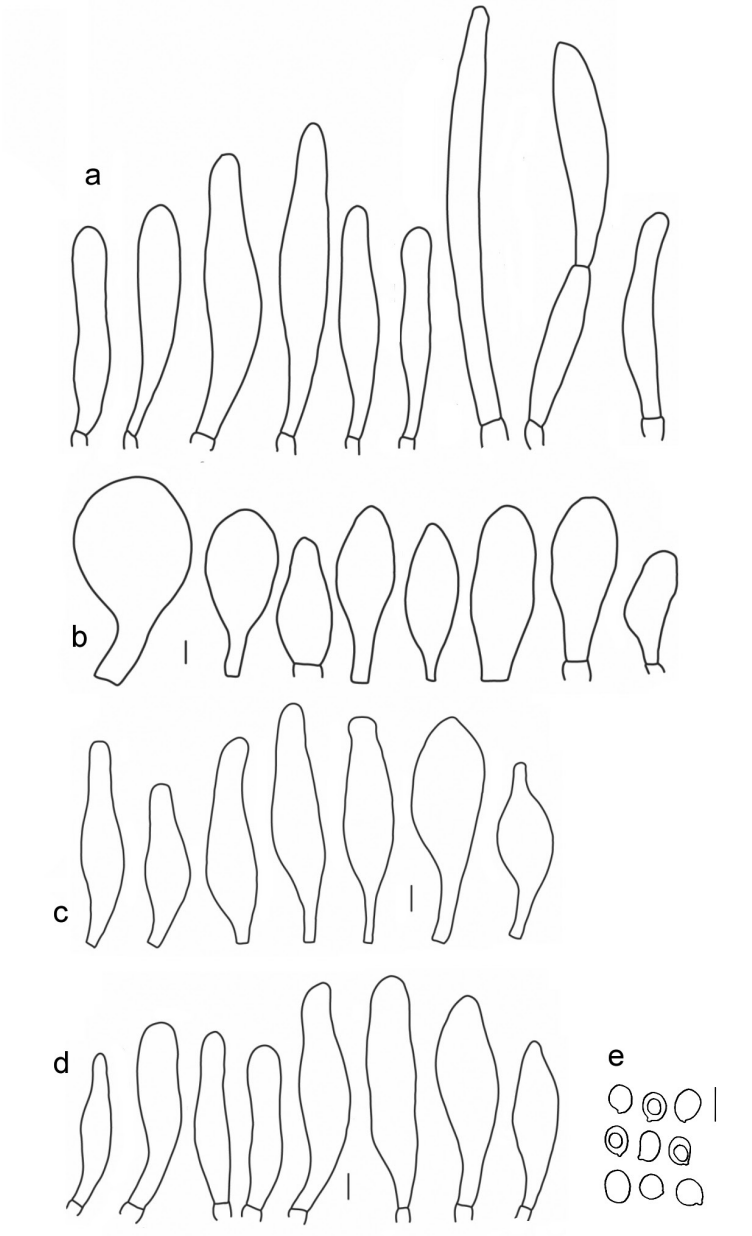


Fig. 5. *Pluteus punctatus* (JHC 04_298): a. pileipellis elements; b. cheilocystidia; c. pleurocystidia; d. caulocystidia. (del. A. Justo). e. spores. (del. H. Ševčíková). Scale bars = 10 μm.

Basidiospores [1/1/30] 6.5–8.5 × 4.5–7.0 µm, avl × avw = 7.3 × 5.8 µm, Q = (1.10-)1.15–1.39, avQ = 1.27, broadly ellipsoid to ellipsoid, rarely subglobose. Basidia 24–32 × 7–11 µm, tetrasterigmate. Pleurocystidia abundant, 50–80(-100) × 17–32(-44) µm, mostly lageniform, narrowly utriform or fusiform; hyaline. Cheilocystidia abundant, 58–106 × 17–46 µm, mostly clavate, narrowly utriform or fusiform; hyaline. Pileipellis a trichoderm, terminal elements 85–250 × 15–27(-35) µm, (narrowly) clavate, narrowly utriform, fusiform, or cylindrical. Caulocystidia abundant, isolated or in loosely arranged clusters; 61–100 × 17–31 µm, narrowly clavate, narrowly utriform, lageniform, or fusiform; hyaline. Clamp connections absent in all studied tissues.

Collection studied: **SWEDEN**, **Halland**, Dömestorp, Musikedalen, Vallåsen, 56°22′17″N 13°05′45″E, on *Fagus* log, 5 X 2004, leg. Jacob Heilmann-Clausen (JHC 04-298, originally as *P.* cf. *depauperatus*).

Comments—The Swedish specimen was collected from a fallen beech log in a small (4.4 ha) almost pure beech stand containing a considerable amount of dead wood, often in sunlit gaps. The ~210-year-old stand is rather even-aged and part of the larger (110 ha) Musikedalen nature reserve, protected since 2010. The stand has been without active management of the living trees since 1946 and under strict protection since 1996 (Länstyrelsen Halland 2010). The forest lies at 180 m a.s.l. adjacent to a larger bog area and elsewhere surrounded by spruce plantations. The climate is temperate with an Atlantic influence with an annual average precipitation of 1000–1100 mm (SMHI 2018a) and ca. 7 °C temperature (SMHI 2018b).

Distribution & ecology

Pluteus dianae was previously known only from its type locality in Czechia. Our study extends the known distribution to Denmark and Russia. In addition, phylogenetically identical collections are reported from Turkey (GenBank Acc. No. MG544918.1 and MG544917.1, as *Pluteus plautus* (Kaygusuz & al. 2019). *Pluteus dianae* can now be said to occur through northern, central, and eastern Europe into Turkish Asia. Climatically, the European collection sites lie in the temperate continental zone (SMHI 2018b, Tolasz 2007) with precipitation averaging 600–900 mm annually (Merkel 2018, Wang 2013). The sites are located from the lowlands to the mountains. The Turkish collections were found near mountains at 250–850 m elevations in northwest Turkey in areas of regular and high rainfall (Kaygusuz & al. 2019). *Pluteus dianae,* which grows on decaying trunks of deciduous trees, is known from *Fagus* spp., *Populus tremula*, and a fallen trunk of an unidentified deciduous tree.

Pluteus punctatus was also known only from its type locality in Czechia. The Swedish collection adds important ecological and distributional data. Its collection sites lie in the European temperate area (SMHI 2018b, Tolasz 2007) with a mean annual precipitation of 504–1100 mm (Merkel 2018, SMHI 2018a). Both sites are located in the lowlands. *Pluteus punctatus,* which grows on dead wood of deciduous trees, has been collected from a *Populus* sp. stump and fallen *Fagus sylvatica* trunk.

Other studied collections of similar taxa:

Pluteus atriavellaneus: **USA**: **Tennessee**, **Unicoi Co.**, Unaka Springs, dead wood, 18–24 Aug. 1904, W. A. Murrill 673 (NY, type).

Pluteus avellaneus: **USA**: **New York**, **Essex Co.**, Lake Placid, Adirondacks, dead wood, 17-29 Jul. 1912, W. A. Murrill 91 (NY, type).

Pluteus compressipes: **JAMAICA**: **Castleton Gardens,** dead wood, wet and shaded, 15 Dec. 1908, W. A. Murrill 118 (NY, type).

Pluteus depauperatus: **FRANCE**: **Château de La Grange,** Yerres, stumps and trunks of *Fagus*, Aug. 1936 leg. & det. H. Romagnesi (PC, type).

Pluteus fibrillosus: **USA**: **Louisiana**, **New Orleans,** Chalmette, New Orleans, in soil in a wet thicket, 8 Sept. 1908, F. S. Earle 129 (NY, Type).

Pluteus fuliginosus: **USA**: **New York**, **Essex Co.**, Lake Placid, Adirondacks, 17-29 Jul. 1912, white pine stump decayed, coniferous or mixed forest, W. A. Murrill 118 (NY, type).

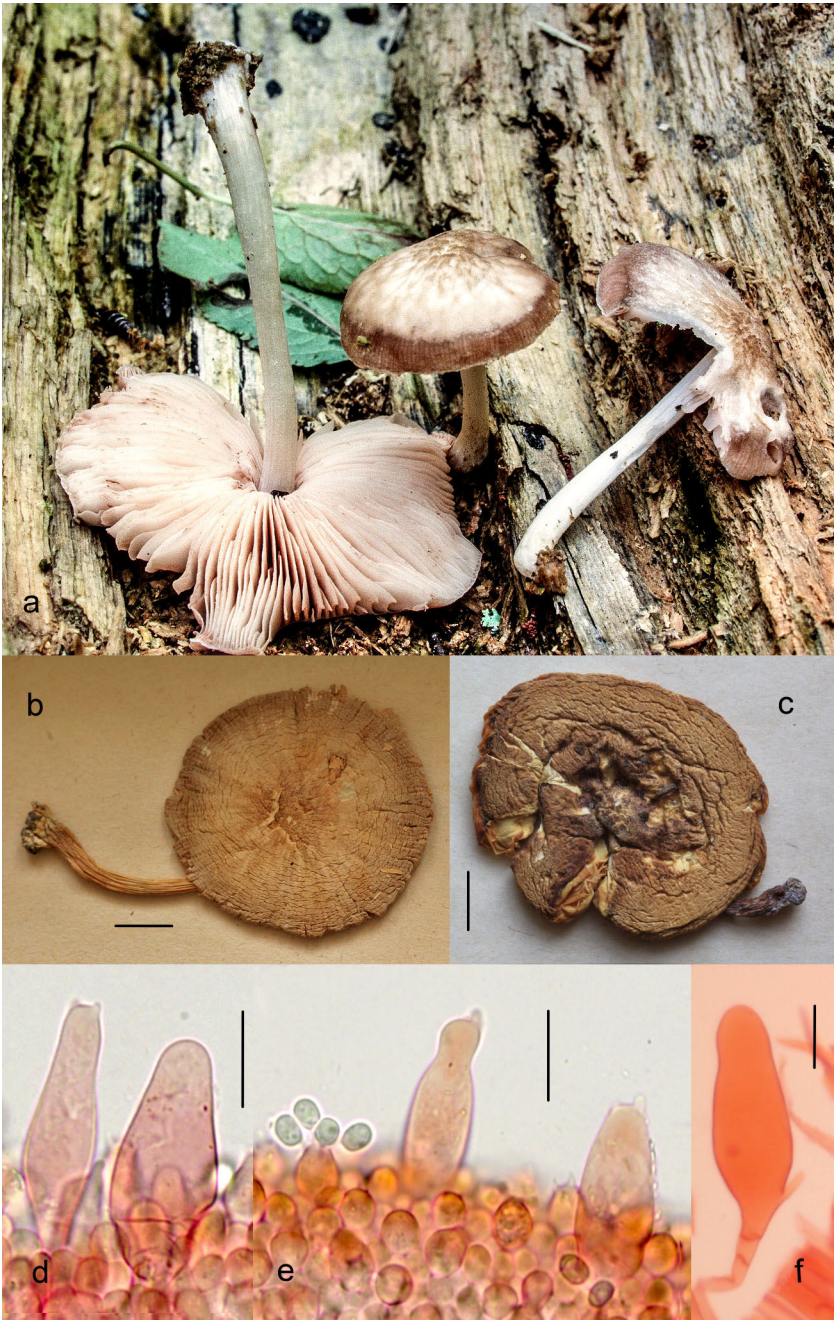
Pluteus granulatus: **CZECHIA: Kladeruby nad Oslavou,** Vlčí kopec, cultivated spruce forest, on a rotten stump of *Picea abies*, 3 Oct. 2013 leg. & det. H. Ševčíková (BRNM 761707); **Česká Třebová,** Křivolík stream, on rotten stump of *Picea abies* lying in a stream, 15 Aug. 2017 leg. M. Mička, det. H. Ševčíková (BRNM 807610); Psáry, cultivated spruce forest, near a road, cavity of rotting stump of *Picea abies*, 20 Sept. 2015 leg. J. Herčík, det. H. Ševčíková (BRNM 807611). **ITALY**, probably **Trentino**, Val di Sole, rotten ? of *Abies* near meadow, Aug. 1882 leg. & det. G. Bresadola (S F14380 lectotype).

Pluteus hiatulus: **FRANCE**: **Oise**, Lamorlaye, rotten deciduous stump (*Populus* sp.?) 10 Sept. 1946 leg. Romagnesi H.? (PC, type); Château de La Grange, Yerres, stumps of *Fagus*, Aug. 1936 leg. & det. H. Romagnesi (PC, herbarium of Romagnesi).

Pluteus inflatus: **CZECHIA**: **Mnichovice,** trunk of *Alnus* (*A. glutinosa* according to observations by H.Š.), Aug. 1944, leg. & det. J. Velenovský (PRM 154569, type).

Pluteus latifolius: **USA**: **Washington**, Seattle, on dead alder, 20 Oct. – 1 Nov. 1911, W. A. Murrill 510 (NY, type).

Fig. 6. ***Pluteus dianae:*** a. (JHC 99_30) basidiomata (photo J. Heilmann-Clausen); b. (holotype, PRM 629413) dried basidioma (photo H. Ševčíková). ***Pluteus punctatus*** c. (holotype, PRM 682743): dried basidioma (photo H. Ševčíková). ***Pluteus dianae:*** (LE312950): d. pleurocystidia; e. basidium with spores and pleurocystidia (photos E.F. Malysheva). f. (JHC 99_30). caulocystidium (photo H. Ševčíková). Scale bars: b–c = 1 cm, d–f = 20 μm.


a
b
c
d
e
f

Pluteus plautus sensu lato: **CZECHIA**: **Babice,** PR Čihadlo, *Fagetum*, fallen stem of *Fagus sylvatica*, 17 May 2017 leg. & det. H. Ševčíková (BRNM 807612, HS9); Valtice, Rendezvous National Nature Monument, fallen deciduous stem, 2017 leg. P. Včelička, det. H. Ševčíková (BRNM 807613, HS18_6).

Pluteus puberulus: **CZECHIA**: **Choteč, Radotín valley**, rotten stump of *Alnus* sp. (probably *A. glutinosa*), Aug. 1918, leg. & det. J. Velenovský (PRC, Velenovský collection, bottle 78 f, type).

General discussion

Taxonomy

Pluteus dianae was originally characterized by its large whitish pileus tinged slightly yellowish-brown at the centre and a whitish striate stipe subpruinose only in the lower part (Pilát 1968). However, all studied Russian collections with similar ITS sequence data are described has having a brown or greyish-brown pileus with a velvety surface or covered with white appressed hairs and a whitish or yellowish striate often granulose-pruinose stipe. Microscopically, Pilát (1968) emphasized the absence of metuloid cystidia with hooks but did not explicitly describe any other kinds of pleurocystidia. Our examination of the holotype (PRM 629413!) revealed (narrowly) lageniform or fusiform pleurocystidia with a narrowed apex and a pedicel (Fig. 2e), also found in all other studied collections (Figs 3e, g; 6d–e). Pilát (1968) cited basidiospores measuring 7.5–9.0 × 5.0–6.5 µm, while our type revision showed a slightly wider range [(6.0–)6.5–8.5(–9.5) × 5.0–6.5(–7.0) µm] in concordance with the Russian collections. The Danish collection is morphologically similar to the type and Russian collections except for the scattered caulocystidia, apparently reflecting the rather poor condition of the dried material, as the macroscopical notes mention the stipe as being whitish floccose.

Although the Spanish collection AJ209 (Justo & al. 2011b) is phylogenetically close to *Pluteus dianae,* we maintain its identification as *Pluteus* aff. *dianae* based on its different ITS sequence and the following morphological differences from *P. dianae*: (i) smaller pilei (3.5–5 cm diam.); (ii) broadly ellipsoid to ellipsoid (rarely subglobose) and smaller basidiospores [5.5–7.5 × 4.4–6.2 µm, avl × avw = 6.6 × 5.3 µm, Q = (1.08) 1.17–1.40, avQ = 1.24]; and (iii) pleurocystidia without apical excrescences.

Pluteus punctatus was described with a pale grey-brown pileus with dark-brown floccose squamules, a whitish stipe with brown tomentose squamules, and variably shaped cystidia (Wichanský 1972: 1). The pileus scales of the Swedish collection are grey-brown in centre, outwards cream to ochraceous. Our microscopical examination of the holotype (PRM 682743!) and

in concordance with the Swedish collection reveals striking fusiform, lageniform, or utriform pleurocystidia ≤80 µm long (Figs 4e, 5c) and caulocystidia not arranged in large tufts but isolated or in loosely arranged clusters (Figs 4f, 5d).

Similar taxa

According to Pilát (1968), *Pluteus dianae* resembles *Pluteus petasatus* (Fr.) Gillet and *Pluteus pellitus* in having a large white pileus. However, these species belong to *Pluteus* sect. *Pluteus*, characterized by metuloid pleurocystidia and a cutis pileipellis.

Both *Pluteus dianae* and *P. punctatus* belong to the taxonomically complicated *P. plautus* complex. Different authors have interpreted the species in this complex in noticeably diverse ways. Whereas Citérin & Eyssartier (1998) mentioned about six species in Europe, Vellinga (1990) recognized only one broad species—*Pluteus plautus*—incorporating nine taxa described by Lasch (Fries 1838), Bresadola (1881, Schulzer 1885), Romagnesi (Kühner & Romagnesi 1953), Orton (1960, 1969), and Wichanský (1972). Other poorly known species of this complex were described by Velenovský (1921) and Pilát (1968). Several additional species in the complex described from North America (Murrill 1917) may well also occur in Europe. Below we provide an overview of names published in this complex in Europe and North America, discussing their similarity to *Pluteus dianae* and *P. punctatus* so as to establish a basis for future taxonomic work in this species complex.

Pluteus plautus (Weinmann 1836: 394), was originally characterized by an alutaceous to fuligineous pileus with a velvety brown to blackish brown stipe and stipe context. Gillet (1876: 394) interpreted it as a small species without specifying basidioma dimensions. *Pluteus punctatus* was originally described by Wichanský (1972) as macroscopically similar to *P. plautus,* but it differs from *P. punctatus* (and *P. dianae*) in having a darker stipe and brown stipe context. Based on Weinmann's type description and our own field experience, we consider the dark colour of the context (especially in the stipe) an important diagnostic character of *P. plautus* sensu stricto. As *P. plautus* is interpreted differently by several agaricologists (e.g. Orton 1986, Vellinga 1990, Citérin & Eyssartier 1998), a neotypification is needed.

Following the tradition of Kühner & Romagnesi (1953), *Pluteus semibulbosus* (Lasch) Quél. also belongs to the *P. plautus* complex. It was originally characterized as a small whitish species with a softly atomate,

sulcate pileus and pubescent stipe with a distinctly bulbous base (Fries 1838). Lasch (in Fries 1838) did not mention basidioma dimensions, but Saccardo (1887: 674) mentioned a 13 mm broad pileus and 25 mm long stipe. Orton (1960: 349, 1986: 55) interpreted *P. semibulbosus* as belonging to *P.* subsect. *Eucellulodermini*, and published the name *Pluteus boudieri* P.D. Orton for *P. semibulbosus* sensu Boudier, Kühner & Romagnesi. However, for us, the original description of the stipe as pubescent strongly supports *P. semibulbosus* in *P.* sect. *Hispidoderma* and within the *P. plautus* complex. This interpretation would place the name *P. boudieri* in synonymy with *P. semibulbosus*. Recent collections referred to *P. semibulbosus* by us demonstrate that this broad morphological species concept corresponds to a polyphyletic group (unpublished data) and that further studies, including the selection of a type for *P. semibulbosus,* are needed. Even in its broad concept *Pluteus semibulbosus* can be distinguished from *P. punctatus* and *P. dianae* at least by its smaller basidiomata and bulbous stipe bases, while pale colouration is more diagnostic of *P. punctatus.*

Pluteus aestivus Velenovský (1921: 607) was described as similar to *P. semibulbosus* but having cuneiform-ellipsoid, non-translucent brown basidiospores, suggesting it belongs in a different genus than *Pluteus*. As original material is no longer extant, the name is impossible to interpret exactly, and we consider it a nomen dubium.

Pluteus candidus Patouillard (1887: 156) was described as a small species with a white, non-striate, silky pileus up to 10 mm broad, a pale thin stipe, and fusiform cystidia. It differs from both *P. dianae* and *P. punctatus* by its smaller basidiomata and silky pileus, and *P. punctatus* is further distinguished by the darker pileus colour. *Pluteus candidus* probably belongs to the *P. semibulbosus* complex. A smooth non-striate pileus is an important diagnostic feature for *P. candidus*. Nonetheless, finding a holotype of *P. candidus* is important for future research.

Pluteus stylobates Velenovský (1921: 608) was described as a small whitish agaric with a ≤10–15 mm broad white pileus with dark squamules and a discoid stipe base resembling *Mycena stylobates* (Pers.) P. Kumm. The species therefore seems to belong to the *P. semibulbosus* complex. Unfortunately the holotype (PRC, Velenovský collection, bottle no. 396), no longer exists (it dried up and was destroyed years ago). This taxon may be a good species, but neotypification is necessary. Both *P. dianae* and *P. punctatus* have larger basidiomata and lack a discoid stipe base, and *P. punctatus* differs also by the darker colours.

Pluteus gracilis (Bres.) J.E. Lange was originally described by Bresadola (Schulzer 1885: 134) as *P. pellitus* var. *gracilis* Bres. Lange (1936: 84) assigned this taxon to *P.* subsect. *Depauperati*. His description and painting (Lange 1936: tab. 71 E) showed a whitish to very pale ochre *Pluteus* with dirty brown squamules on the pileus and stipe, presumably belonging to the *P. plautus* complex. However, it is unclear based on which material Bresadola described this taxon as a variety of *Pluteus pellitus*. Until a type has been located, we find it difficult to interpret this taxon.

Pluteus depauperatus Romagn. (Kühner & Romagnesi 1956: 181) was described with an 18–50 mm broad, velvety dull pale brown or brownish grey-ochre pileus, distinctly striate at the margin and sometimes granulate in the centre. The stipe was described as white when young, but striped pruinose brown-saffron to brick-saffron in mature specimens (Kühner & Romagnesi 1953). The holotype of *P. depauperatus* (PC!) contains two envelopes with material. The envelope with the larger basidiocarp marked as the type is in a rather poor condition, with deformed or collapsed cystidia and irregular, probably deformed, basidiospores (7.0–8.0 × 4.0–5.0 µm). The envelope with the smaller basidiocarp is better preserved and its microcharacters agree with Romagnesi's original description (Kühner & Romagnesi 1953) and spores measuring (5.0–) 6–6.5 (–7.0) × 5.0–6.0 µm, avl × avw = 6.33 × 5.43 µm. Pilát (1968), who mentioned the similarity of *P. dianae* with *P. depauperatus,* distinguished the latter by its pale brown, grey-brown or saffron-brown pileus, smaller basidiospores (see above), and an odor resembling *Lepiota cristata* (Bolton) P. Kumm. or *Scleroderma* Pers. The saffron-tinged mature stipe should also help separate *P. depauperatus* from both *P. dianae* and *P. punctatus.* Moreover, in all observed collections, *P. punctatus* lacks a striate pileus margin. *Pluteus depauperatus* seems to represent a good species, but verification by holotype DNA sequencing is needed.

Pluteus hiatulus Romagn. (Kühner & Romagnesi 1953: 421, as nom. nov., validly published in 1956: 182 with the addition of the Latin diagnosis) was originally described as having a dirty brown, soon fading pileus with a *Coprinus*-like membranaceous striate margin, a satin white to pale brown (more at the base) stipe, and refractive utriform or lageniform pleurocystidia with pale brown vacuoles Our holotype revision (PC!) confirmed that the pleurocystidia are refractive, narrowly to broadly (sub)lageniform to fusiform (rarely subutriform to utriform), and only rarely with very pale brown guttules. However, it is possible that brown guttulae fade with time

in exsiccates. Romagnesi's measurements (in Kühner & Romagnesi 1953) of subglobose basidiospores at 5.7–6.2(-7.5) × 5–6.5 µm agree with our type study. *Pluteus dianae* and *P. punctatus* differ from *P. hiatulus* by having a thicker-fleshed pileus with a less striate and non-*Coprinopsis*-like margin and possessing hyaline non-refractive pleurocystidia.

Pluteus punctipes Orton (1960: 361) was described as having relatively small to moderately large basidiomata with a sepia or umber to vandyke brown coloured pileus and sepia to dirty yellowish brown minute fibrillose scales on both pileus and stipe. Its possession of large (≤100 × 30 µm) lageniform pleurocystidia with a short to long neck and with a brown vacuole is macroscopically expressed in some places as slightly dark brown lamellae. Orton (1960) also mentioned narrow clamped hyphae below cylindrical-fusiform pileipellis elements. The species seems to be close to *P. plautus* s. str. *Pluteus dianae* and *P. punctatus* differ in their hyaline and shorter pleurocystidia. Further studies and sequencing of the type are needed to clear up the taxonomic position and delimitation of *P. punctipes*.

Pluteus dryophiloides Orton (1969: 115) was originally described as having colours similar to *Gymnopus dryophilus* (Bull.) Murrill with a whitish or buff coloured stipe; a pileipellis with relatively short (60–152 µm) terminal cells; and vesiculose-fusiform, broadly pyriform, or broadly lageniform pleurocystidia sometimes terminating in solitary short pimples. *Pluteus dianae* and *P. punctatus* differ by their duller pileus colours, longer pileipellis terminal elements, and different pleurocystidia.

Pluteus granulatus Bresadola (1881: 10) was described as having a brown-reddish granulate pileus and a whitish, finely furfuraceous stipe with a yellowish furfuraceous base; the species was described as growing on coniferous wood, and that substrate may indeed be an important character for separating *P. granulatus* from other species in the *P. plautus* group. We consider collections HS11 (BRNM 807610) and HS18_2 (BRNM 807611) (Fig. 1) to represent *P. granulatus* in its original sense. Unfortunately, Kühner & Romagnesi (1956) cited *P. granulatus* as growing on *Fagus sylvatica*, which caused subsequent taxonomic confusion. In GenBank, several collections assigned to *Pluteus granulatus* were indicated as growing on *Fagus orientalis*. Our ITS sequences (HS9 and HS18_6), molecularly similar to other collections named *P. granulatus* from *Fagus sylvatica,* do not represent *P. granulatus*. These collections are here referred to *Pluteus* sp. VI (Fig. 1) pending further clarification of the *P. plautus* complex. *Pluteus dianae* and *P. punctatus* differ from *P. granulatus* by growth on deciduous wood and the

lack of a reddish tinge and distinct granules on the pileus, with the pileus of *Pluteus dianae* usually paler. Microscopically, the lectotype (S F14380!) of *P. granulatus* possesses lageniform (rarely fusiform) pleurocystidia that lack the apical excrescences, which are relatively frequent in *P. dianae*. Further, the pileipellis terminal elements are shorter and more uniform in size than in *P. punctatus* and *P. dianae*.

Pluteus puberulus Velenovský (1921: 607) was described as having a leathery, ochre, brown-streaked pileus that in places is almost squamulose, scaly, entirely ochre, or covered by small grains and a stipe that is grooved, densely velvety pubescent, and dirty yellowish. Velenovský (1921) also described yellowish cheilocystidia. The holotype is preserved in a bottle of Velenovský's solution (Fassatiová & al. 1994) in which yellow colours were said to fade (Velenovský (1921: 608). As the holotype (PRC, Velenovský collection, bottle 78 f!) contains only a part of the pileus in bad condition, determination of the identity of the preserved fungus is impossible and the taxonomic position of *P. puberulus* remains unclear.

Pluteus opponendus (Britzelm.) Sacc., originally described with a white pileus and stipe, might resemble *P. dianae* except for the significantly different basidiospores (8–9 × 4 µm; Britzelmayr 1881: 136). *Pluteus opponendus* has an unclear taxonomical position.

Pluteus tiliaceus Velenovský (1921: 607), which was described with white to whitish colours similar to *P. opponendus*, is unique within the *P. plautus* complex by its small (~4 µm diam.) globose basidiospores, smooth, glabrous, radially fibrillose pileus, and clavate cystidia (Velenovský 1921). The original material does not exist, and the taxonomical value and position of *P. tiliaceus* remains unclear.

Pluteus praestabilis (Britzelm.) Sacc. was originally characterized by a blackish brown velvety pruinose pileus, a white stipe with brown floccules, basidiospores measuring 6 × 4–5 µm, and growth on soil near *Fagus sylvaticus* (Saccardo 1887: 672). Although it probably belongs to the *P. plautus* complex, *P. praestabilis* may also represent a species related to *P. podospileus* Sacc. & Cub. *Pluteus dianae* and *P. punctatus* have larger basidiospores, with *P. dianae* further distinguished by a stipe without brown floccules and a paler pileus.

Vellinga & Schreurs (1985) cited *Pluteus inflatus* Velen. as a synonym of *P. plautus*. However, our study of the holotype (PRM 154569!) showed that its pileipellis is composed of sphaeropedunculate and long elements, and pleurocystidia are absent. Our microscopical examination combined

with the macroscopic description by Velenovský (1921: 609) suggests that *P. inflatus* is closer to *P. podospileus* than to *P. plautus*.

The North American *Pluteus longistriatus* (Peck) Peck is characterized by a cinereous or whitish pileus, with striations that reach the darker, minutely squamulose-hairy disc, and a glabrous white stipe (Peck 1885). All these characters separate *P. longistriatus* from *P. dianae* and *P. punctatus.* Molecular data (Fig. 1) also support recognition of *P. longistriatus* as a separate taxon. Morphologically, *P. longistriatus* resembles the European *P. hiatulus*. Further phylogenetic and morphological research is needed to clarify the relationship between *P. longistriatus* and *P. hiatulus*.

As part of a project aiming to clarify the taxonomy of the North American *Pluteus* species, A. Justo revised the type collections of the 46 species described by Murrill (1911, 1917, 1939, 1943, 1946). Six belong to the *P. plautus* complex: *P. atriavellaneus* Murrill, *P. avellaneus* Murrill, *P. compressipes* Murrill, *P. fibrillosus* Murrill, *P. fuliginosus* Murrill, and *P. latifolius* Murrill. *Pluteus dianae* differs microscopically from them mainly by differently shaped pleurocystidia (only *P. latifolius* possesses pleurocystidia commonly bearing apical excrescences similar to *P. dianae*). *Pluteus punctatus* differs microscopically from the North American taxa mainly by its predominantly clavate to narrowly utriform cheilocystidia. *Pluteus latifolius* was originally described from Washington state, USA, growing on dead *Alnus* wood (Murrill 1917). Its pileus is avellaneous-isabelline, tomentose with a radiate-rugose centre, and ≤4 cm broad; the stipe is concolorous with the pileus and densely tomentose (Murrill 1917). *Pluteus latifolius* differs from *P. dianae* in its smaller basidiomata, smaller basidiospores (5.2–7.2 × 4.5–5.5 µm, avg. = 6.2 × 4.8 µm, Q = 1.18–1.39, avQ = 1.29), and lageniform caulocystidia.

Pluteus atriavellaneus was described from Tennessee, USA, growing solitary on humus (Murrill 1917). In contrast to *P. dianae*, it is a small species (pileus ≤2 cm) with a dark fuliginous to avellaneous, hygrophanous and finely pubescent pileus and white, smooth stipe (Murrill 1917). *Pluteus punctatus* differs from *P. atriavellaneus* by the presence of squamules on pileus and stipe.

Pluteus avellaneus was described from New York, USA, growing on unidentified dead wood (Murrill 1917). The pileus is described as small, hazel coloured, paler at centre, glabrous, hygrophanous and ≤3.5 cm broad; the stipe is white and smooth (Murrill 1917). Its smaller pileus and stipe dimensions and hazel color of the smooth pileus distinguishes *P. avellaneus* from *P. dianae. Pluteus punctatus* differs from *P. avellaneus* by the presence

of squamules on pileus and stipe. Neither *P. punctatus* nor *P. dianae* have a hygrophanous pileus.

Pluteus compressipes is a tropical species from Jamaica, found growing on unidentified dead wood (Murrill 1917). Its pileus is rosy-isabelline with a darker centre, glabrous, and ≤4 cm broad and its stipe is white, smooth, and laterally compressed (Murrill 1917). Its occurrence in tropical areas and a smooth compressed stipe are significant differences from *Pluteus dianae* and *P. punctatus*.

Pluteus fibrillosus, collected in Louisiana, USA, from wet soil was characterized by a dark, innately fibrillose, ≤3 cm broad pileus and smooth white stipe (Murrill 1917). *Pluteus dianae* and *P. punctatus* differ by growth on decaying wood and a (sub)flocculose stipe. The pileus of *P. punctatus* is not innately fibrillose but floccose-squamulose, and the pileus of *P. dianae* is paler and larger.

Pluteus fuliginosus, from New York, USA and collected from a decayed *Pinus strobus* stump, was described with a uniformly coloured (fuliginous) pileus clothed with white hairs and ≤4 cm broad and a white to pale yellow or pale hazel and slightly squamulose stipe (Murrill 1917). The *P. fuliginosus* stipe was, as in *P. punctatus*, originally described as squamulose, although we observed no caulocystidia on the type collection. The cheilocystidia of *P. fuliginosus* are mostly lageniform or fusiform, and commonly provided with an apical excrescence (A. Justo, pers. obs.). Its habitat on conifer wood may be a reliable character to separate *P. fuliginosus* from other species in this complex, but additional collections are necessary to confirm this.

Conclusions

Our analyses confirm *Pluteus dianae* and *P. punctatus* as separate, molecularly supported species. We characterize *P. dianae* by rather large basidiomata, a whitish to greyish-brown pileus that may turn pale after drying, and a striate granulose-pruinose whitish stipe. Its striking microscopic features are its lageniform and fusiform pedicellate pleurocystidia with a narrowed apex often bearing a small apical excrescence, variable sized pileipellis elements, and caulocystidia in tufts.

Pluteus punctatus is characterized by medium sized basidiomata, a pale grey-brown pileus with ochraceous or (grey-) brown floccose squamules, and a whitish stipe with brown tomentose squamules. Microscopically striking are the rather short pleurocystidia, the long terminal elements in the pileipellis and caulocystidia that are isolated or occur in only small tufts.

Collections of *P. dianae* from central, eastern, and northern Europe show that this species is widespread in Europe. Moreover, it has also been documented from Turkey. The *P. punctatus* material of from Sweden is the only collection besides the holotype from Czechia. The distribution of both species should be further investigated.

Acknowledgments

The authors thank J. Holec (National Museum, Herbarium PRM, Prague, Czech Republic) and N. Menolli Jr. (Instituto Federal de Educação, Ciência e Tecnologia de São Paulo (IFSP), Brazil) for their pre-submission reviews of the manuscript. The authors also thank A. Anderberg (S); E. Bloch (NY); A. Bond (K(M)); B. Buyck (PC); J. Holec (PRM), O. Koukol (PRC), and M. Smith (FLAS) for the possibility to study herbarium collections, M. Mička and J. Herčík for their collections, and M. Sochor for sequencing some collections. We also thank J.W. Jongepier who helped improve the language of the manuscript. The studies by Hana Ševčíková were enabled by support provided to the Moravian Museum by the Ministry of Culture of the Czech Republic as part of its long-term conceptual development programme for research institutions (DKRVO, ref. MK000094862). The studies of the Ekaterina F. Malysheva were enabled by support provided to project AAAA-A19-119020890079-6. Ekaterina F. Malysheva's research was funded by a project of the Komarov Botanical Institute of the Russian Academy of Sciences, and Michal Tomšovský's research was supported by the European Regional Development Fund, Project *Phytophthora* Research Centre, Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000453.

Literature cited

Bresadola G. 1881 [reprint 1976]. Fungi Tridentini 1. J. Zippei Edit, Tridenti.

Britzelmayr M. 1881. Hyporhodii und Leucospori aus Südbayern (Hymenomyceten aus Südbayern 2). Berichte des Naturhistorischen Vereins Augsburg. 26: 133–148.

Citérin M, Eyssartier G. 1998. Clé analytique du genre *Pluteus* Fr. Documents Mycologiques 28(111): 47–67.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. https://doi.org/10.1093/nar/gkh340

Fassatiová O, Klán J, Prášil K, Skalický V, Urban Z. 1994. Houby. in: Křísa B, Prášil K. (eds.). Sběr, preparace a konzervace rostlinného materiálu, PřF, UK Praha.

Fries E. 1838. Epicrisis Systematis Mycologici. Typographia Academica, Upsaliae.

Gardes M, Bruns T. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Gillet CC. 1876. Les Hyménomycètes ou Description de tous les Champignons qui Croissent en France. Ch. Thomas, Alençon.

Justo A, Vizzini A, Minnis AM, Menolli N Jr, Capelari M, Rodríguez O, Malysheva E, Contu M, Ghinone S, Hibbett DS. 2011a. Phylogeny of *Pluteaceae* (*Agaricales*, *Basidiomycota*): taxonomy and character evolution. Fungal Biology 115: 1–20. https://doi.org/10.1016/j.funbio.2010.09.012

Justo A, Minnis AM, Ghignone S, Menolli N, Capelari M, Rodríguez O, Malysheva E, Contu, M, Vizzini A. 2011b. Species recognition in *Pluteus* and *Volvopluteus* (*Pluteaceae*, *Agaricales*): morphology, geography and phylogeny. Mycological Progress 10(4): 453–479. https://doi.org/10.1007/s11557-010-0716-z

Kaygusuz O, Türkekul İ, Knudsen H, Çolak Ö. 2019. New records of *Pluteus* section *Hispidoderma* in Turkey based on morphological characteristics and molecular data. Phytotaxa. 413. 175–206. https://doi.org/10.11646/phytotaxa.413.3.1

Kornerup A., Wanscher JH. 1983. Methuen Handbook of Colour. 3rd ed. London, Eyre Methuen.

Kühner R, Romagnesi H. 1953. Flore Analytique des Champignons Supérieurs. Masson et cie, Paris.

Kühner R, Romagnesi H. 1956. Compléments à la "Flore analytique". VIII. Espèces nouvelles, critiques ou rares de Volvariacées. Bulletin de la Société Mycologique de France. 72(3): 181–249.

Lange JE. 1917. Studies in the Agarics of Denmark. Part III. *Pluteus*, *Collybia*, *Inocybe*. Dansk botanisk Arkiv. 2 (7): 1–50.

Lange JE. 1936. Flora Agaricina Danica 2. Copenhagen.

Länstyrelsen Halland. 2010. Skötselplan för naturreservatet Musikedalen i Laholms kommun. http://www.lansstyrelsen.se/halland/SiteCollectionDocuments/Sv/djur-och-natur/skyddad-natur/naturreservat/laholm/Musikedalen-skotselplan.pdf

Malysheva E, Malysheva V, Krasilnikova A. 2009. Morphological and molecular study of the genus *Pluteus*. Mikologia i Fitopatologia 43: 216–231.

Malysheva E, Malysheva V, Justo A. 2016. Observations on *Pluteus* (*Pluteaceae*) diversity in South Siberia, Russia: morphological and molecular data. Mycological Progress 15: 861–882. https://doi.org/10.1007/s11557-016-1215-7

Menolli Jr N, Justo A, Capelari M. 2015. *Pluteus* section *Hispidoderma* in Brazil with new records based on morphological and molecular data. Cryptogamie Mycologie 36(3): 331–354. https://doi.org/10.7872/crym/v36.iss3.2015.331

Merkel A. 2018. Climate: Snina. https://en.climate-data.org/location/7246/ (accessed 2 May 2018).

Murrill WA. 1911. The *Agaricaceae* of tropical North America: IV. Mycologia 3(6): 271–282.

Murrill WA. 1917. (*Agaricales*), *Agaricaceae* (pars), *Agariceae* (pars). North American Flora 10: 77–144.

Murrill WA. 1939. Additions to Florida fungi–I. Bulletin of the Torrey Botanical Club 66 (1): 29–37. https://doi.org/10.2307/2481013

Murrill WA. 1943. Some southern novelties. Mycologia 35(4): 422–433. https://doi.org/10.2307/3754594

Murrill WA. 1946. New and interesting Florida fungi. Lloydia 9(4): 315–330.

Nikolcheva LG, Bärlocher F. 2004. Taxon-specific fungal primers reveal unexpectedly high diversity during leaf decomposition in a stream. Mycological Progress 3: 41–49. https://doi.org/10.1007/s11557-006-0075-y

Orton PD. 1960. New check list of British Agarics and Boleti, part III (keys to *Crepidotus*, *Deconica*, *Flocculina*, *Hygrophorus*, *Naucoria*, *Pluteus* and *Volvaria*). Transactions of the British Mycological Society 43(2): 159–439.

Orton PD. 1969. Notes on British Agarics III. Notes from the Royal Botanical Garden Edinburgh 29(1): 75–127.

Orton PD. 1986. British Fungus Flora. Agarics and Boleti 4: *Pluteaceae*: *Pluteus* and *Volvariella*. Royal Botanic Garden, Edinburgh.

Pilát A. 1968. *Pluteus dianae* sp. nov. bohemica subsectionis *Depauperati* Lange. [*Pluteus dianae*, nový druh štítovky z podsekce *Depauperati* Lange.] Česká Mykologie 22(3): 171–173.

Pradeep CK, Justo A, Vrinda KB, Shibu VP. 2012. Two new species of *Pluteus* (*Pluteaceae*, *Agaricales*) from India and additional observations on *Pluteus chrysaegis*. Mycological Progress 11(4): 869–878. https://doi.org/10.1007/s11557-011-0801-y

Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014. Tracer v1.6. Accessed on 20.12.2017: http://tree.bio.ed.ac.uk/software/tracer/

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology. 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Saccardo PA. 1887. Sylloge hymenomycetum, vol. I. *Agaricineae*. Sylloge Fungorum 5. 1146 p.

Schulzer S. 1885. Einige neue Pilz-Species und Varietäten aus Slavonien. Hedwigia 24(4): 129–151.

Singer R. 1986. The *Agaricales* in modern taxonomy. 4th edn. Koeltz Scientific Books, Koenigstein.

Skalický V. 1988. Regionálně fytogeografické členění. 103–121, in: S Hejný, B Slavík (eds.), Květena České socialistické republiky 1. Academia, Praha.

SMHI. 2018a. Normal uppskattad årsnederbörd, medelvärde 1961-1990. Assessed May 2018: http://www.smhi.se/klimatdata/meteorologi/nederbord/normal-uppskattad-arsnederbord-medelvarde-1961-1990-1.6934

SMHI. 2018b. Normal årsmedeltemperatur. Assessed May 2018: http://www.smhi.se/klimatdata/meteorologi/temperatur/normal-arsmedeltemperatur-1.3973

Syrový S. 1958. Atlas podnebí ČSR. Ústřední správa geodézie a kartografie, Praha

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Thiers B. 2018 [continuously updated]. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Accessed 9 April 2018: http://sweetgum.nybg.org/ih/

Tolasz R. 2007. Atlas podnebí Česka. 1. vydání. Český hydrometeorologický ústav, Universita Palackého, Praha, Olomouc.

Vellinga EC. 1988. Glossary. 54–64, in: Bas C, Kuyper TW, Noordeloos ME, Vellinga EC. (eds.). Flora agaricina neerlandica, vol. 1. A.A. Balkema, Rotterdam, Brookfield.

Vellinga EC. 1990. *Pluteaceae* Kotl. and P. 31–56, in: Bas C, Kuyper W, Noordeloos ME, Vellinga EC. (eds.). Flora agaricina Neederlandica, vol. 2, Balkema, Rotterdam.

Vellinga EC, Schreurs J. 1985. *Pluteus* Fr. in West Europe. Persoonia 12(4): 337–373.

Weinmann JA. 1836. Hymeno- et Gastero-mycetes hucusque in imperio Rossico observatos. Inpensis Academiae Imperialis Scientiarum, Petropoli.

Velenovský J. 1921. České houby. Česká botanická společnost, Praha.

Wang PR. 2013. DMI Teknisk Rapport 12-23 Referenceværdier: Måneds- og årskort 2001–2010, Danmark for temperatur, relativ luftfugtighed, vindhastighed, globalstråling og Nedbør.

White TJ, Bruns T, Lee S, Taylor JW. 1990. 38—Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: Innis MA, Gelfand DH, Sninsky JJ, White TJ. (eds.). PCR Protocols: A Guide to Methods and Applications. Academic Press, Inc., New York. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

Wichanský E. 1972. Štítovka tečkovaná – *Pluteus punctatus* Wich. n. sp. Časopis Českých Houbařů 49(1–2): 1.

Zahradnický J, Mackovčin P. 2004. Chráněná území ČR. XI., Plzeňsko a Karlovarsko. Agentura ochrany přírody a krajiny ČR. AOPK ČR, EkoCentrum Brno.